Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 61; Numer/Number 2/2010
ISSN 0423–104X

Does the response of GH-secreting pituitary adenomas to octreotide depend on the cellular localization of the somatostatin receptor subtypes SSTR2 and SSTR5?

Czy odpowiedź gruczolaków przysadki wydzielających hormon wzrostu na oktreotyd zależy od komórkowej lokalizacji podtypów SSTR2 i SSTR5 receptora somatostatynowego?

Hanna Pisarek[1], Marek Pawlikowski[1], Jolanta Kunert-Radek[2], Katarzyna Winczyk[1]

[1]*Department of Neuroendocrinology, Medical University, Łódź*
[2]*Department of Clinical Endocrinology, Medical University, Łódź*

Abstract

Introduction: The immunohistochemical examination of somatostatin receptor (SSTR) subtypes expression in different endocrine tumours, including pituitary adenomas, revealed membranous or cytoplasmic distribution of SSTR1–5. Currently used long-acting somatostatin analogue octreotide prefers SSTR2 and SSTR5 subtypes. In an earlier study a positive correlation between the summarized score of SSTR2A + SSTR2B expressions and growth hormone (GH) response to octreotide administration was found, independently of receptor distribution within the cell. In this study we searched for the relationship between the GH inhibitory response to acute octreotide administration and SSTR2A, SSTR2B, and SSTR5 cellular localization.
Material and methods: Thirteen acromegalic patients underwent a test of acute administration of octreotide before surgery. The drop in GH was defined as the percentage of the basal value. The pituitary adenomas from these patients were immunostained to determine the hormonal phenotype and expression of SSTR subtypes. The subcellular distribution pattern of SSTR subtypes — membranous or cytoplasmic — was determined.
Results: In the majority of specimens, cytoplasmic localization of receptor subtypes was observed, although membrane or mixed cytoplasmic-membranous localized immunopositivity also occurred. The drop in GH after octreotide administration varied between 57.1–96.7% (mean 82.1%). Among the patients with the cytoplasmic localization of SSTR5, the decrease in GH was significantly higher (92.0 ± 7.0%). A tendency towards the higher response in patients with cytoplasmic localization of SSTR2A and 2B was also observed (86.8% and 87.0%, respectively).
Conclusions: It seems that cytoplasmic localization of SSTR5, SSTR2A, and SSTR2B is connected with enhanced GH inhibition after octreotide administration. It is possible that this somatostatin analogue alters the localization of subtypes SSTR2A and SSTR2B through the receptor internalization. As a consequence, the SSTR-immunopositivity in cell cytoplasm is increased. The cytoplasmic but not the membranous localization is connected with the higher responsiveness to the octreotide in *somatotropinomas.*
(Pol J Endocrinol 2010; 61 (2): 178–181)

Key words: *octreotide, somatostatin receptor subtypes, somatotropinoma*

Streszczenie

Wstęp: Badania immunohistochemicze ekspresji podtypów receptora somatostatynowego (SSTR1–5, *somatostatin receptor 1–5*) w guzach endokrynnych wykazują ich obecność zarówno w błonie komórkowej, jak i na terenie cytoplazmy komórek guza. Szeroko stosowany obecnie syntetyczny analog somatostatyny — oktreotyd wykazuje powinowactwo głównie do podtypów SSTR2 i SSTR5. W naszych wcześniejszych badaniach zauważono pozytywną korelację między zsumowanym wskaźnikiem (*score*) nasilenia odczynu immunohistochemicznego SSTR2A i 2B, a hamowaniem sekrecji hormonu wzrostu (GH, *growth hormone*) po podaniu oktreotydu u chorych z akromegalią, niezależnie od lokalizacji receptorów w komórkach guza. Obecne badania autorów pracy dotyczą ewentualnej współzależności pomiędzy hamowaniem GH w odpowiedzi na oktreotyd a komórkową lokalizacją SSTR2A, 2B i 5.
Materiał i metody: Zbadano 13 chorych z akromegalią, u których przed operacją wykonano krótki test z oktreotydem. W gruczolakach przysadki określono immunohistochemicznie ich fenotyp hormonalny oraz występowanie i lokalizację podtypów SSTR.
Wyniki: W większości przypadków obserwowano cytoplazmatyczną ekspresję badanych podtypów SSTR, aczkolwiek występowała również lokalizacja błonowa i mieszana błonowo-cytoplazmatyczna. Spadek wydzielenia GH w teście hamowania oktreotydem zawierał się w przedziale pomiędzy 57,1–96,7% (średnio 82,1%). Największy znamienny spadek wydzielenia GH (92,0 ± 7,0%) po podaniu oktreotydu wystąpił u pacjentów z cytoplazmatyczną lokalizacją SSTR5 w komórkach guza. Zauważono także tendencję do silniejszej odpowiedzi u pacjentów, u których również SSTR2A i 2B miały położenie cytoplazmatyczne (odpowiednio 86,8% i 87,0%).
Wnioski: Wydaje się, że cytoplazmatyczne, a nie błonowe położenie SSTR2A, 2B i 5, jest powiązane z silniejszym hamowaniem GH w teście z oktreotydem. Możliwe, że oktreotyd w procesie internalizacji receptora zmienia jego położenie z błonowego na cytoplazmatyczne i zwiększa jego immunopozytywność w cytoplazmie badanych komórek guza.
(Endokrynol Pol 2010; 61 (2): 178–181)

Słowa kluczowe: *oktreotyd, podtypy receptora somatostatynowego, guzy somatotropowe przysadki*

The project was supported financially by the Medical University of Łódź, No 503-5084-1.

Hanna Pisarek Ph.D., Department of Neuroendocrinology, Medical University of Łódź, Sterling St. 3, 91–425 Łódź, tel.: +48 42 632 48 31, fax: +48 42 636 54 27, e-mail: h.pisarek@kardio-sterling.lodz.pl

Introduction

The immunohistochemical investigation of somatostatin receptor subtypes (SSTR) expression in different endocrine tumours, including pituitary adenomas, revealed membranous or cytoplasmic distribution of SSTR1–5 within the investigated tumour cells. As the SSTR belongs to a family of seven transmembrane domains linked with the G protein receptors [1, 2], one could conclude that only the membrane-localized immunostaining is compatible with the functional role of somatostatin receptors. Earlier experience supports such a thesis [3–5]. Therefore, some authors suggest that in neuroendocrine tumours, only the membranes located SSTR2 are active although the active SSTR3 and SSTR5 subtypes posses the cytoplasmic localization [6, 7]. On the other hand, in earlier studies [8–11] we described membranous, cytoplasmic, and mixed membranous-cytoplasmic distribution of SSTR1–5 in thyroid, adrenal, and pituitary tumours as well as neuroendocrine tumours, without the statement of their functional state. There is a highly variable expression of SSTR subtypes in these tumours. This fact may partially explain why some of them (for instance a subgroup of *somatotropinomas* or other pituitary adenomas) do not respond to the therapeutic action of the currently used long-acting somatostatin analogues: SSTR2 and SSTR5, preferring octreotide and lanreotide. A significant positive correlation between the summarized score of SSTR2A + SSTR2B expressions and growth hormone (GH) response to octreotide administration was found, independently of receptor distribution within the cell [12]. In this study a tendency towards a positive correlation between the summarized score of SSTR2A + SSTR2B + SSTR3 + SSTR5 to the octreotide treatment was also observed. However, the interdependence of the octreotide effect and somatostatin receptor subtype SSTR2 and SSTR5 distribution was not studied. So in the present study we would like to take into consideration the possible relationship between GH inhibition level to acute octreotide administration and SSTR2A, SSTR2B, and SSTR5 localization in *somatotropinoma* cells.

Material and methods

A group of 13 acromegalic patients (9 females and 4 males, aged 23–67 years, mean age 43.4 years) was investigated. Before surgery, all of the patients underwent a test of acute administration of octreotide (Sandostatin, Novartis) given subcutaneously in a dose of 200 μg. The growth hormone level was measured in the blood serum at the time intervals 0′, 60′, 120′, and 240′ after injection. The drop in GH was defined as the percentage of the basal value. The pituitary adenomas removed by transsphenoidal adenomectomy from these patients were fixed in Bouin-Hollande fixative and paraffin embedded. All samples were immunostained with specific mono- and polyclonal antibodies directed to pituitary hormones or α subunits to determine the hormonal phenotype of the adenomas. For somatostatin receptor subtype determination, the immunohistochemical procedure was performed as previously described [13]. The 4–5 μm paraffin sections were immunostained using commercially available rabbit polyclonal antisera raised against carboxyl-terminal fragments of specific human somatostatin receptor subtypes (GRAMSCH Laboratories, Schwabhausen, Germany). The immunoreactive intensity for specific receptor proteins was scored semiquantitatively using a descriptive scale as follows: strong staining (+++), moderate staining (++), weak staining (+), and trace staining (±). Subcellular distribution pattern of SSTR subtypes — membranous or cytoplasmic — was also determined. Statistical analysis was performed using Statistica 8 software. The level of statistical significance was set at $p < 0.05$.

Results

In all the investigated tumours the immunohistochemical estimation of hormonal phenotype revealed positive immunostaining of GH. In all but one tumour, PRL was also co-expressed. In the three adenomas, co-expression of LH was found, and another two adenomas co-expressed TSH or free α-SU. All of the adenomas investigated expressed the somatostatin receptor subtypes with different intensity. Only SSTR4 did not occur in any of investigated samples. We found strong and moderate immunostaining of SSTR2A in 9/13 adenomas (69.2%). In the remaining 4 cases the reaction was weak or weak to moderate. Somatostatin receptor 2B was expressed in the same score 9/13 (69.2%). In the remaining 4 patients the reaction was trace, weak, or weak to moderate. The immunopositivity of SSTR5 with strong and moderate grade of intensity appeared in 10/12 (83.3%) of the tumours. In the two adenomas the reaction was weak to moderate. We also determined the immunoreaction for the remaining subtypes: SSTR1 (10/12 = 83.3%) and SSTR3 (9/12 = 75%). In the majority of specimens cytoplasmic localization of receptor subtypes was observed, although membrane or mixed cytoplasmic-membranous localized immunopositivity also occurred. The drop in GH after the octreotide administration varied between 57.1% and 96.7% (mean 82.1%). Thus, all patients were "responders". Table I presents the percentage of GH in dependence to the cellular distribution of the investigated subtypes of SSTR: 2A, 2B, and 5. As can be seen, the decrease in GH in response to the octreotide is significantly higher in the case of

Table I. *The correlation between the inhibition of growth hormone (GH) in response to acute octreotide treatment (expressed in percentage rate) and SSTR2A, SSTR2B, and SSTR5 distribution in the somatotropic pituitary adenomas*

Tabela I. *Współzależności pomiędzy hamowaniem hormonu wzrostu (GH) w odpowiedzi na krótki test działania oktreotydu a komórkową lokalizacją SSTR2A, 2B i SSTR5 w somatotropowych guzach przysadki*

SSTR subtype	Percentage (%) of response GH to acute octreotide treatment with membranous receptor localization	Percentage (%) of response GH to acute octreotide treatment with cytoplasmic receptor localization
SSTR2A	76.3 ± 16.95	87.0 ± 13.95, $p > 0.05$ (NS)
SSTR2B	78.9 ± 17.2	86.8 ± 13.1, $p > 0.05$ (NS)
SSTR5	66.4 ± 11.6	92.0 ± 7.0, $p < 0.001$
SSTR2A+2B+5	73.5 ± 15.4	88.6 ± 11.2, $p < 0.01$

patients in whom the purely cytoplasmic distribution of SSTR5 was found. There is also a tendency towards a higher response in patients with cytoplasmic localization of SSTR2A and 2B.

Discussion

The pattern of SSTR expression in acromegaly (as estimated according to the percentage frequency of appearance with strong to moderate intensity of staining) was SSTR5 = SSTR1 > SSTR3 > SSTR2A = SSTR2B. It agrees with our earlier observations [10] in which the dominance of SSTR5 and, to a lesser degree, that of SSTR1 was found in *somatotropinomas*. In the group of 12 patients where SSTR5 was estimated, this subtype with cytoplasmic distribution was observed in seven patients and with mixed membranous-cytoplasmic localization in five patients. This membranous-cytoplasmic distribution was accompanied by a lower mean level of GH inhibition after acute octreotide administration, compared with SSTR2A and SSTR2B (see Table I). On the other hand, the cytoplasmic localization of this receptor subtype revealed the strongest mean GH inhibition in this test. A similar tendency with strongest GH inhibition after acute octreotide treatment was found in cases of SSTR2A and SSTR2B localized in the cytoplasm. Only 3 out of 13 patients demonstrated strong or moderate intensity of immunohistochemical staining of SSTR2A with membranous or mixed membranous-cytoplasmic distribution. The other six specimens exhibited evidently only cytoplasmic localization of SSTR2A with this grade of intensity. This fact did not support the view that SSTR2 subtype is the integral membranous form of somatostatin receptor and the rapid targeting and integration of this receptor subtype into the cell membrane is responsible for the failure of its detection in the cytoplasm. Moreover, it seems that, as in the case of SSTR5, only cytoplasmic SSTR2A and SSTR2B were connected with enhanced GH inhibition after acute octreotide treatment. Consequently, it is possible that this somatostatin analogue alters the localization of both subtypes 2 (2A and 2B) of somatostatin receptor through the receptor internalization and consequently increases the SSTR-immunopositivity in cell cytoplasm. The cytoplasmic localization of immunoreactive receptor proteins may be explained in several ways. Firstly, it may be the result of receptor internalization under the influence of an agonist, as discussed above. Secondly, it may represent the receptor protein *de novo* synthesized within the endoplasmic reticulum. Lastly, we cannot exclude that this localization is an artefact produced during the fixation procedure. Summing up, the data presented above, taken together with our earlier study [12], show that the response of GH-secreting adenoma to octreotide is linked with both the intensity of SSTR immunostaining (which represents the total amount of immunoreactive receptor protein) and the SSTR cellular localization, but, paradoxically, the cytoplasmic and not membranous localization is connected with higher responsiveness.

References

1. Reisine T, Bell G., Molecular Biology of Somatostatin Receptors. Endocrine Reviews 1995; 16: 427–442.
2. de Herder WW, Hofland LJ, Van der Lely AJ et al. Somatostatin Receptors in gastroenteropancreatic neuroendocrine tumours. Endocrine-Related Cancer 2003; 10: 451–458.
3. Pawlikowski M. Somatostatin Receptors in Human Tumors- In Vitro Studies. In: *Somatostatin Analogs in Diagnostics and Therapy*. Edited by Marek Pawlikowski, Landes Bioscience 2007; 39–46.
4. Reubi JC, Waser B, Liu Q et al. Subcellular Distribution of Somatostatin sst2A Receptors in Human Tumors of the Nervous and Neuroendocrine Systems: Membranous Versus Intracellular Location. The Journal of Clinical Endocrinology & Metabolism 2000; 85: 3882–3891.
5. Schonbrunn A. Somatostatin receptors — present knowledge and future directions. Annales of Oncology 1999; 10 (Suppl. 2): S17–S21.
6. Volante M, Brizzi MP, Faggiano A et al. Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. Modern Pathology 2007; 20: 1172–1182.
7. Faggiano A, Volante M, Brizzi MP et al. Somatostatin receptor immunohistochemistry in neuroendocrine tumors: a proposal of scoring system for clinical characterization and therapy selection. Endocrine Abstracts 2007; 14: P168.

8. Pisarek H, Stępień T, Kubiak R et al. Somatostatin receptors in human adrenal gland tumors - immunohistochemical study. Folia Histochemica et Cytobiologica 2008; 46, 3: 251–257.
9. Pisarek H, Stępień T, Kubiak R et al. Expression of somatostatin receptor subtypes in human thyroid tumors: the immunohistochemical and molecular biology (RT-PCR) investigation. Thyroid Research 2009; 2: 1.
10. Pisarek H, Pawlikowski M, Kunert-Radek J et al. Expression of somatostatin receptor subtypes in human pituitary adenomas — immunohistochemical studies. Polish Journal of Endocrinology 2009; 60: 240–251.
11. Pisarek H, Pawlikowski M, Kunert-Radek J et al. SSTR 1 and SSTR 5 subtypes are the dominant forms of somatostatin receptor in neuroendocrine tumors. Folia Histochemica et Cytobiologica — manuscript nr: FHC-D-09-00029 R1 2010; 48 (in print).
12. Pawlikowski M, Pisarek H, Kunert-Radek J et al. Somatostatin receptors in GH-secreting pituitary adenomas — their relationship to the response to octreotide. Polish Journal of Endocrinology 2008; 59: 196–199.
13. Schulz S, Schulz St, Schmitt J et al. Immunocytochemical Detection of Somatostatin Receptors sst1, sst2A and sst3 in Paraffin — embedded Breast Cancer Tissue Using Subtype — specific Antibodies. Clinical Cancer Research 1998; 4: 2047–2052.